1. Purpose:

To provide uniform Ion Trap System (ITS) operating and confirmation guidelines to all participants in the USDA/AMS Pesticide Data Program (PDP).

2. Scope:

This SOP shall be followed by all analytical laboratories which are conducting residue studies for PDP. This includes laboratories conducting stability and other studies which may impact PDP.

3. Outline of Procedure:

- General Ion Trap Detection (ITD) procedures and recommendations
- Checking Gas Chromatographic/Ion Trap Detection (GC/ITD) system
- ITS performance check with Decafluorotriphenylphosphine (DFTPP)
- Ion Trap confirmation criteria of analytes

4. References:

- Multiresidue Pesticide Analysis by Ion Trap Technology, Cairns, T., Siegmund, E., Navarro, D., Kin S. Chiu, (in press) Presented at 9th PDP Meeting Herndon, Va.

- Use of Mass Spectrometry for Confirmation of Animal Drug Residues, Sphon, A. James., J. Assoc. Off. Anal. Chem ., 1978, 61, 1247

- Handbook of Mass Spectra of Environmental Contaminants, Hites, A. Ronald; CRC Press Inc, Boca Raton, Florida

- Evolving Criteria for Confirmation of Trace Level Residues in Food and Drugs by Mass Spectrometry, Thomas Cairns, Emil Siegmund, and John Stamp., Mass Spectrometry Reviews 1989, 8, 93-117 & pp 127-145

5. Specific Procedure:

These operating procedures are presented as general guidelines in Electron Impact (EI) mode for total ion current (TIC) profile only. They suggest only the minimum requirements. Each laboratory shall have written operating procedures which shall provide specific details concerning how these procedures have been implemented in that laboratory.

Safety:

All chemicals used in procedures for pesticide analysis should be treated as a potential health hazard, and exposure to chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals, particularly pure standard materials and stock solutions, which should be handled with suitable protection to skin and eyes.

5.1 Apparatus, Materials and Maintenance:

Gas Chromatograph/Ion Trap System

- A temperature programmable gas chromatograph, complete with all accessories, and equipped with ITD. The injection port must be designed for capillary column injections, with the other end of the capillary placed in the ion source for maximum sensitivity. The injection system must not be allowed to contact metal surfaces that promote decomposition.

- Any bonded phase fused silica capillary column, which fosters good chromatography can be used with the gas chromatograph. A 30 meter column with a film thickness of 0.25 micron may be used but a film thickness of 1.0 micron is recommended because of its larger capacity.

- The ion trap system must be equipped with 70 volts electron energy in the EI mode, capable of scanning 35-500 amu (atomic mass units; daltons) with a complete scan cycle time. The GC/ITD system must produce a mass spectrum, which meets the recommended or a modified version of calibration criteria listed in section 5.3. To ensure a safer environment, the effluents from sample splitters of GC and vacuum pumps of ITS shall be vented or absorbed on an activated charcoal column.

- All maintenance performed on the instrument shall be documented in the instrument log book. The septums, injection port liner, guard column, solvent, and syringes will be replaced as necessary or as warranted by the chromatography. Follow the direction in the manual for routine ion trap maintenance of the detector and vacuum pumps.

- A computer system shall be interfaced to the ion trap system that allows it to acquire and store mass spectral data obtained throughout the duration of the chromatographic program. The software must be able to recognize the GC peak within a given

retention time window and have library search option of the acquired data.

5.2 Reagents, Calibration and Quality Control

- Use of known standards of the analytes is required for the mass spectral analysis. Their concentration generally corresponds to the detection limit of the method used and the tolerance level of the pesticide. The standards shall be checked periodically for any signs of degradation or evaporation and replaced immediately, if perceptible changes are noticed in their response. The standards shall be prepared fresh at least every six months. All standard solutions shall be stored at not less than -20 degrees Celsius in amber, Teflon-lined screw cap, bottles.

- Calibrate the mass and abundance scales of the ion trap system daily in accordance with procedures prescribed by the manufacturer to assure the performance of the ion trap. Make adjustments and modifications necessary to meet the requirements. Verify the calibration at the beginning of each day and every eight hours.

- Prepare an ITS performance check solution of 5 ng/ul in methylene chloride of DFTPP. Store this in an amber vial in a dark cool place. Inject 1 ul of DFTPP solution and acquire a spectrum that produces a narrow symmetrical peak. If the spectrum does not meet the established criteria (Table 1) or its modified version, retune and adjust the instrument to meet the requirement. The criteria is used to evaluate the performance of the GC/ITD system. Verify the calibration at the beginning of each operational day and every eight hours within a run.

- Efficiency of the column should be checked by the extent of separation of either a mixture of phenanthrene and anthracene, or benz[a]anthracene and chrysene. In the first case the separation of the two compounds is affected at the baseline, while in the later case the height of the valley should be less than 25% of the average peak height of the two compound. A pesticide may also be used for checking the efficiency of the column.

- Before any samples are analyzed, it should be demonstrated that laboratory reagent blank is reasonably free of contamination that would prevent the determination of the analyte of concern. In general, background from method analytes should be below the method detection limit. If reagent or matrix broad background interference restricts the sensitivity of the GC/IT analysis, additional cleanup procedures (e.g., florisil column, Sep pack, Gel Permeation etc.,) are recommended, especially for samples with pesticides at low ppb concentration level. Matrix blank, and fortified

matrix samples should be analyzed to meet other quality control requirements. It is appropriate to demonstrate the absence of matrix effects by comparing detection limit acquired separately from matrix fortified samples and fortified reagent blanks.

- Use of process control standards to monitor variability in retention times and sensitivity are suggested but not required. If a surrogate analyte is used, the relative retention time (RRT) or retention time (RT) shall be the same as stipulated in the method of analysis. If the mass spectrum contains less than the required number of structurally characteristic ions (section 5.6), different confirmation procedures such as chemical ionization (CI, positive or negative), Gas Chromatographic Atomic Emission Detector (GC/AED) shall be used. Process control standard (s) is (are) used as retention time marker (s) and generally added to each sample extract prior to analysis by GC/ITS.

5.3 GC/ITS System Performance Check

- Demonstrate and document an acceptable initial calibration (tune-up). This requirement must be met before any samples are analyzed. Verify the calibration at the beginning of each operational day and every eight hours within a run.

- At the beginning of each day that ion trap confirmations will be performed, the GC/ITS system shall be evaluated against the background corrected spectrum of DFTPP. For GC/IT confirmatory work, GC/ITS must meet the established criteria (Table 1) with any modification. If the criteria is not met, retune and/or perform preventive maintenance on the instrument to meet the requirement. The criteria is used to evaluate the performance of the GC/ITD system.

Table 1. Ion Abundance Criteria for DFTPP (5 ng)

Mass	Relative Abundance Criteria
51	10-80% of the base peak
68	< 2% of mass 69
70	< 2% of mass 69
127	10-80% of the base peak
197	< 2% of mass 198
198	198 or base peak 0r > 50% of 442
199	5-9% of mass 198
275	10-60 % of mass 198

365	> 1% of mass 198
441	Present but less than 443
442	Base peak or 50% of 198
443	15-24% of mass 442

Sometimes ions do not fall within the range defined in Table 1. Establish a historical profile of range within which to expect the relative percent abundance for ions of DFTPP under 100. Replace this range as a criteria for ions under 100 amu in Table 1.

The response and retention time of the daily DFTPP must be evaluated before sample analysis begins. If the response is less than 80% of the reference response (i.e., the response obtained when detection limits are measured), the sensitivity of the GC/ITS system must be inspected for possible deterioration, and corrective action shall be taken (e.g., increase electron multiplier voltage, replace leaky septum, etc). If the retention time changes by more than 10 percent from the previous standard, the chromatographic system must be inspected for malfunctions and corrective action shall be taken.

5.4 Confirmation Detection Limit

- Each laboratory shall, establish the confirmation detection limit of each analyte in every commodity being analyzed. Since the objective is to verify the positive residue findings in commodities, obtained by other analytical techniques in the laboratory, a minimum confirmation detection limit which is equal to or below the detection limit of the method of analysis shall be demonstrated by each laboratory. If the ion trap cannot achieve the method detection limits, alternate confirmation procedures such as chemical ionization (CI, positive or negative) or Gas Chromatography Atomic Emission Detector (GC/AED), shall be used, to sufficiently demonstrate confirmation.

5.5 Operation Procedures

EI Mode

- The ion trap should be tuned in EI mode daily in accordance with the procedure described in the Operator's Manual. Perform air-water check for acceptable ratios of water and nitrogen ions and adjust calibration gas levels as stated in the manual. For sample analysis, set parameters for optimum sensitivity and analyze the whole set of samples at these parameters.

5.6 Confirmation Guidelines

- An injection of the standard prepared in solvent, at approximately five times the minimum level of detection or at approximately the same concentration as the analyte in the sample, is made to generate a standard reference spectrum. The spectrum should be background-subtracted. The same GC/ITS operating conditions must be used for subsequent samples analysis.

- Generally five or a minimum of three most characteristic ions with significant relative abundance (include no more than one chlorine cluster ion, with a chlorine cluster treated as one ion in case of polychlorinated compounds) are a sufficient number for comparison with the ions in the samples.
- All structurally significant ions of the reference spectrum must exhibit intensities of at least three times signal to noise (3xS:N). Ions that are chosen as structurally significant ions for confirmations must have intensities greater than 10% of the most abundant ion. If the presence of a molecular ion is indicated in the spectrum, it must be incorporated as one of the identifying ions. If possible, the selected ions should have a molecular weight greater than 90. For a confirmation, a minimum of 3 ions is required. If a compound gives only two ions with their structure directly related to the compound, then two ions will be sufficient confirmation of residues. If this requirement of minimum number of ions is not met, findings may not be reported as "MS Confirmed". Structural isomers that produce similar spectra can be explicitly identified only if they have sufficiently different GC retention times. Otherwise structural isomers are identified as isomeric pairs.

- A matrix blank shall be analyzed with each set of confirmation samples to demonstrate the absence of contamination in the laboratory. If contamination is suspected, all samples associated with that matrix blank must be reanalyzed.

- Compare the mass spectrum of the sample with positive response to the standard reference spectrum or a suitable spectrum from a reference library.

5.7 Confirmation Criteria of Residues

- A presumptive positive residue in the sample is confirmed by satisfying two criteria for the verification of identification:

  (1) The GC RRT or RT of the presumptive positive sample component falls within 0.01 units or 10 secs. of the RRT or RT respectively of the compound of interest and

of the fortification standard. In some situations the mass spectrometer operators will have to use their professional judgment to confirm a residue sample.

(2) The mass spectra of the sample component matches the mass spectrum of the daily standard or mass spectra of the reference library (percent fit profile be within the scope of the chemist's judgement).

- Ions present in the standard spectrum but not initially obvious in the sample spectrum should be verified by subtracting responses due to contaminated background from the sample spectrum.

- Ions present in the standard spectrum but not initially obvious in the sample spectrum should be verified for possible deletion from the sample spectrum because their intensities are below the data acquisition baseline threshold.

- When comparing spectra, the relative abundance of the standard and sample component should be considered (i.e. minor ions in the high abundance spectrum may not be observed in the low abundance spectrum).

- The relative intensities of ions shall agree within +/- 15% in the full scan mode between standard and sample, except for possible deviations that can be verified by the reasons stated above.

- Data review and assessment requires a laboratory chemist with experience in mass spectrometry operations and identification, who has the approval of the laboratory director.

- Document the ITS confirmation method, GC/ITD EI or CI(scan) etc. as described in 5.8 below.

5.8 Documentation

The following documents shall be kept as part of confirmation QA/QC.

- Chromatograms for air or water as a means to ascertain vacuum for the system, thereby its sensitivity.

- Daily tuning profiles of DFTPP or other compounds as well as logs of all instrument maintenance such as source cleaning, replacement of inserts,

septums, and columns.

- A log of all samples injected and type of compounds confirmed with appropriate operating parameters.

- Copies of chromatograms of samples, standards, sample blanks and reagent blanks.

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